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HYDRA VIRIDIS: TRANSFER OF METABOLITES BETWEEN *HYDRA* AND SYMBIOTIC ALGAE

GLYNE THORINGTON AND LYNN MARGULIS

Department of Biology, Boston University, Boston, MA 02215

Abstract

"Back transfer" of metabolites from food to endosymbiotic algae in the digestive cells of *Hydra viridis* was demonstrated. Brine shrimp nauplii labeled with tritiated precursors of protein and nucleic acids (DNA and RNA) were fed to light and dark grown hydras. The fate of the label after a single feeding with radioactive material in hydra and algal fractions was followed by scintillation counting and autoradiographic techniques. Labeled thymidine was incorporated into DNA in both light- and dark-grown hydras. Although the symbiosis persists indefinitely in hydras in darkness (7–10 days) the number of algae per cell is reduced.

Tritiated orotic acid and tritiated uridine, RNA precursors, were incorporated into the RNA fraction of hydra cells and algae. Tritiated leucine was incorporated into peptides and proteins, and to a lesser extent into simple sugars, oligosaccharides, and oligonucleotides in hydra and algal fractions. Thus the metabolites of the brine shrimp food are available to both partners.

A decrease over time in label introduced as ^{3}H-orotic acid and ^{3}H-uridine and incorporated into hydra RNA is compensated for by an increase in label in the algae, implying competition for constant quantities of metabolites from the single feeding. Although food availability, light, number of algae per cell, and other factors influence the quantity and rate of nutrient transfer between the partners, in both light and dark grown hydras the amount of "back transfer" of metabolites to the symbiotic algae is impressive.

Introduction

Green hydras, first reported in the 18th century, have been studied continually since Geddes (1882). All the many hydra strains isolated harbor characteristic algae of the genus *Chlorella*. These green hydras, *Hydra viridis* (synonyms in the literature include *Chlorohydra viridis* and *Hydra viridissima*), taken from freshwater ponds and streams all over the world, are easily maintained in the laboratory. They are kept in the light, fed crustaceans such as brine shrimp every 2 or 3 days, and must be carefully cleaned to prevent overgrowth of bacteria. Because the hydra is disassociable from its *Chlorella* sp. symbionts, *H. viridis* is a preferred laboratory organism for research on metabolic and growth relationships in symbiosis (Muscatine, 1967; Muscatine and Lenhoff, 1965a, b; Cook, 1972; Pardy, 1974). As in other invertebrate-algal endosymbioses, the algae, restricted to the gastrodermal (endodermal) cells of the hydras, transfer photosynthate to their hosts (Muscatine and Hand, 1958; Goreau and Goreau, 1960; Lenhoff and Muscatine, 1963). White, aposymbiotic hydras, rare or unknown in nature, occasionally have been observed in recently hatched hydras but can be produced routinely by treatment with high

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light intensities in the presence of chemical inhibition of photosynthesis (Pardy, 1976).

A large volume of literature of the past decade deals with the metabolic relationships between freshwater hydras and their algal symbionts, with emphasis on the benefits to the host (Muscatine and Lenhoff, 1965b; Muscatine, 1967; Cernichiari *et al.*, 1969; Pardy and White, 1977). Most of the biochemical studies have been concerned with unidirectional movement of metabolites from autotroph to heterotroph. For example, in green hydras allowed to incorporate $^{14}CO_2$ photosynthetically (Muscatine and Lenhoff, 1965b) the algae-free ectoderm accumulated approximately 10–12% of the total fixed ^{14}C at a rather constant rate over 48 h. The specific activity of the ectodermal tissues from the green hydras was much higher than that of the aposymbiotic controls, suggesting a net translocation of fixed radioactive carbon to the host ectoderm.

Isolated symbiotic algae release about 85% of total fixed carbon, mainly as the disaccharide maltose (Muscatine, 1965). The nature of the algal metabolite excreted is influenced by the pH of the medium: Maltose is the primary product excreted at pH 4.5, but it decreases as the medium becomes more alkaline, with little or no maltose released at neutral pH (Cernichiari *et al.*, 1969). Traces of glucose, alanine, glycolic acid, and an unknown oligosaccharide also are excreted. Maltose thus is thought to be the major nutrient released to the host. The ^{14}C translocated to the animal host is detected in the animal tissue as small molecules, nucleic acids, alcohol soluble peptides, proteins, and glycogen (Roffman and Lenhoff, 1969). About 35% of the labeled carbon is present in hydra tissue as glycogen after 4 h incubation.

This movement of photosynthate seems to be of great survival value to hydras when food is limited. As long as illumination is adequate, starved green hydras survive for months, far longer than their aposymbiotic counterparts (Muscatine and Lenhoff, 1965a, b; and unpublished observations of ours and D. C. Smith, Oxford University). The algae's contribution to hydra growth efficiency (percent of energy, consumed as food, converted to new protoplasm; Slobodkin, 1962) increases in starved animals (Stiven, 1965). Although algae probably contribute organic materials that enhance the host's growth and survival, the algae probably do not affect growth by producing oxygen or removing carbon dioxide, since aposymbiotic hydras do not need algae when food is available.

The first sign of a reverse flow of metabolites was obtained when ^{14}C labeled brine shrimp were fed to hydras (Cook, 1972) in either constant darkness or constant light. After 48 h in the light, from 22% to 26% of the recovered activity was in the algal fraction, whereas in the dark 25% to 34% was in the algal fraction. Thus, in both light and darkness, chlorellae take up carbon from the hydra's ingested food. Furthermore, nutrients move from hydras to algae, because algae are retained in well-fed hydras kept in continuous darkness.

To verify further a bidirectional flow of nutrients, Cook (1972) fed ^{35}S-labeled food to *Aiptasia* sp., and separated the algal fraction from the host tissue. He found that algae take up and incorporate radioactive label into protein rather quickly, and retain about 15–30% of the label for at least three to five days.

The experiments reported here were designed to determine the nature of "back transfer" and to verify that it occurs regularly. Hydras were fed once with labeled brine shrimp and the fate of the label monitored in animal and algal fractions by scintillation counting. To locate the label, hydra cells and isolated algae were studied by autoradiography using tritiated precursors of protein, DNA, and RNA.

A large number of gram-negative rod-shaped bacteria also have been found in

symbiotic relationship with green hydras (Margulis *et al.*, 1978; Wilkerson, 1980). In the Ohio hydra strain, used in most of the present studies, the bacteria are in vacuoles of the same gastrodermal cells in which the *Chlorella* reside. The bacteria have been cultivated in pure culture and identified as members of the species *Aeromonas punctata* (Berger *et al.*, 1979). The aeromonads are closely associated with the algal symbionts throughout the symbionts' life cycle (Thorington, 1980). However, intracellular bacteria are absent in the Carolina strain, and English hydras have as-yet-unidentified bacteria at the junctions of the ectodermal cells. Wilkerson (1980) verified that bacteria influence the metabolic activities of the host. She discovered that green hydras kept bacteria-free by antibiotic treatment take up 55% less labeled orthophosphate than green hydra controls with a normal complement of symbiotic bacteria. Our hydras were not treated to remove bacterial symbionts; however, the bacteria were about equally distributed between the animal and algal fractions (light microscopic observations). Hence their quantitative role was ignored.

Materials and Methods

Collection and maintenance of hydras

Two different strains of *H. viridis* were used. The Carolina strain, obtained from the Carolina Supply Co., was used in only one early set of experiments. Because of their large size and sturdiness in culture, all later experiments were done on Ohio strain animals (a gift from Dr. C. Cook, Ohio State University).

Hydras were grown in mass cultures according to the methods of Lenhoff and Brown (1970). Before experiments, all hydras were cultured in "M" solution (Muscatine and Lenhoff, 1965a) at 20° ± 1°C, kept under constant illumination, and fed every other day to repletion on freshly hatched *Artemia* nauplii.

Experimental animals

Only hydras bearing one bud (hydranth) were used for experiments. They were placed in finger bowls and starved for 2 days before experimentation. Sixty hydras were used for incorporation studies involving precursors of protein and RNA. Since H^3-thymidine uptake into DNA is less than into RNA and protein precursors, 210 hydras were used for DNA experiments. During continuous-light experiments, the hydras were placed in an incubator at 20° ± 1°C, with light supplied by two cool white fluorescent bulbs 4–6 in from the hydras. For dark experiments, the finger bowls were wrapped in aluminum foil, placed in a cupboard at 22° ± 2°C, and exposed to no more than 2 min of light during feeding or cleaning.

Incubation of brine shrimp with radioisotopes

Freshly hatched *Artemia* nauplii were placed in small bowls containing 50 ml filtered seawater. Bacterial contamination was reduced by adding 50 mg streptomycin and 165,000 units of penicillin (Sigma Biochemical Co.). To this incubation medium, we added ^{3}H-leucine (specific activity, 60 Ci/m*M*), ^{3}H-thymidine (specific activity, 60.4 Ci/m*M*), ^{3}H-uridine (specific activity, 24.2 Ci/m*M*) or ^{3}H-orotic acid (specific activity, 11.1 Ci/m*M*). The final concentration of radioactivity in the medium in each case was: leucine 0.7 μCi/ml, thymidine 0.4 μCi/ml, uridine 1.0 μCi/ml, and orotic acid 0.3 μCi/ml. After 48 h incubation the nauplii were collected and thoroughly washed with "M" solution. They were then fed to the experimental

hydras. The culture solution was changed after one h, again at six h, and, for longer experiments, daily to lower microbial contamination.

Separation of algae from animal tissue

At intervals after feeding, hydras were removed, washed in ice-cold "M" solution, and homogenized in a glass tissue homogenizer. The suspension was then centrifuged at 300 rpm for 5 min in an International Clinical Centrifuge. The cloudly white residue containing the animal tissue was removed and stored. The green algal pellet (Muscatine, 1965b) was washed several times with "M" solution until a clear residue was obtained. The washings were added to the original animal fraction to give a total volume of 3 ml. A smear of the algal pellet on a slide showed minimal animal-tissue contamination. The algal cells were broken by sonication in ice-cold "M" solution for 2 min (30 s of sonication at 1500 cycles followed by 30 s of cooling, twice). The sonicate was brought to a total volume of 3 ml.

Assay of tritiated leucine into protein

The disrupted tissue, hydra and algae, was brought to a final volume of 3 ml by adding "M" solution. From this, 0.4 ml samples were removed for assay. To 1 ml of the suspension we added 1 ml of ice-cold 10% trichloroacetic acid (TCA). This stood at room temperature for about 15 min. A second 1 ml portion was similarly treated with hot 10% TCA and then placed in a 90°C water bath for 30 min. The TCA-soluble fractions were separated from the TCA-insoluble fraction by filtering the suspension through a 0.45 μm (pore size) Millipore filter. The soluble fractions were collected in a clean test tube. A 0.2 ml portion was removed from the soluble fractions for counting of radioactivity. The insoluble fractions were washed three times with 5% TCA before the filter paper discs were air-dried and then counted.

To 0.6 ml of the cold TCA-soluble fraction we added 3.2 ml of 95% ethanol. This was heated at 45°C for 45 min. Three ml of this suspension was filtered as above to obtain TCA-alcohol soluble and insoluble fractions. From the TCA-alcohol soluble fraction, 1 ml portions were used for radioactive counting. The TCA-alcohol insoluble fractions were washed several times with 80% ethanol before counting.

We added 3.2 ml of 95% ethanol to 0.6 ml of the original suspension. This was then incubated at 45°C for 45 min. Alcohol-soluble and insoluble fractions were obtained by filtering (0.45 μm/Millipore) 3 ml of the suspension. One-ml samples of the alcohol soluble fraction were used for radioactive counting. Alcohol insoluble fractions were washed with 80% ethanol before counting.

In these experiments the distribution of radioactivity was recorded as follows: TCA-soluble, ethanol-soluble fraction contains small molecules such as amino acids and monosaccharides; TCA-soluble, ethanol-insoluble fraction contains oligosaccharides and oligonucleotides; TCA-insoluble, ethanol-soluble fraction contains lipid and lipid soluble components, most small proteins and peptides; and finally TCA-ethanol and hot TCA-insoluble fraction contains the rest of the proteins.

Extraction and assay of radioactivity in RNA

RNA was extracted from the animal and algal fractions by the hot-phenol extraction method of Scherer and Darnell (1962). Distilled phenol was equilibrated with distilled water overnight to give a final concentration of approximately 87% (v/v). One ml of this solution was added to 1 ml of homogenate (hydra fraction)

or sonicate (algal fraction) containing 200 mg of yeast RNA (Sigma Biochemical Co.). After vigorous shaking, the mixture was incubated at 65°C for 10 min, cooled with tap water, and centrifuged at 600 *g* for 5 min at room temperature. The lighter aqueous phase was removed with a Pasteur pipette and an equal volume of phenol solution added. We repeated this extraction sequence three times. After the final centrifugation, two volumes of cold 95% ethanol (pre-chilled at −17°C) were added to the aqueous phase. After quick mixing the solution was kept at −17°C overnight to let RNA precipitate.

On the following day, the material was centrifuged at 7000 *g* for 10 min at 4°C. The ethanol was decanted and the RNA pellet resuspended in 4 ml ethanol. The washing sequence was repeated until we detected no phenol odor. The pellet resuspended in 2 ml 0.01 *M* Tris-HCl buffer, pH 7.4, containing 0.01 *M* NaCl and 0.02 *M* $MgCl_2$. This solution was centrifuged at 10,000 *g* for 15 min, and the supernatant retained to assay radioactivity in RNA.

Tritiated RNA was measured by the disc method (Yu and Feigelson, 1971). We applied 200 ml portions of RNA to filter paper discs (Whatman 3MM, 2.3 cm diameter), air dried the discs, and held them in freshly prepared 10% TCA at 0°C for 3 h to "fix" the sample. The 10% TCA then was decanted and the discs were washed three times with 5% TCA at 0°C to remove acid soluble radioactivity. They were then divided into two groups, of two sample discs and two blanks each. One group was left in cold 5% TCA solution and the other incubated for 20 min at 90°C. All discs were then washed three times with cold 5% TCA, twice extracted with ethanol:ether 1:1 (v/v) at 37°C, and dried under a lamp. Radioactivities of the discs were determined by counting in 10 ml liquid scintillation fluid in a Packard Tricarb Counter.

Enzyme treatment was performed to verify that radioactivity was actually in RNA. The extraction procedures were repeated but to the nucleic acid precipitate was added McIlvaine's citrate phosphate buffer containing RNase (Sigma Biochemical Co.) to give a final concentration of 1 mg/ml at pH 6.7. The sample was incubated at 37°C for 2 h. After incubation the procedure continued as outlined above.

Extraction and assay of radioactivity in DNA

DNA was extracted from the animal and algal fractions by the following method: to 2 ml of homogenate or sonicate containing 200 mg of salmon DNA-carrier was added 1 ml cold 0.6 *N* perchloric ($HClO_4$) acid. This suspension was placed in an ice bath for 10 min and then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was discarded. The pellet was washed twice with 5 ml cold 0.2 *N* perchloric acid. The washes were decanted and the tube allowed to drain onto filter paper for a few minutes. The pellet, with 4 ml of 0.3 *N* potassium hydroxide added, was incubated in a shaking water bath at 37°C for 1 h, and then chilled in an ice bath for 15 min. We then added 0.6 *N* perchloric acid and centrifuged the tubes at 10,000 rpm for 10 min at 4°C. This supernatant was retained. The pellet was washed twice with 5 ml of 0.2 *N* perchloric acid. After another centrifugation as above, the supernatant was retained. The three supernatants were combined and brought to a final volume of 50 ml with distilled water. This fraction contained the RNA. The precipitate was dissolved in 5 ml of 0.3 *N* potassium hydroxide, incubated in a shaking water bath overnight, and the next day diluted to 15 ml with distilled water to obtain the DNA fraction.

To verify that the radioactivity was actually in DNA, the procedure was re-

peated but the precipitates incubated in buffered DNase solutions (Sigma Biochemical Co., 1 mg/ml) for 2 h at 37°C. Equal portions were counted in a toluene-based liquid scintillation fluid.

Autoradiographic experiments

To study the incorporation of label into nucleic acids (RNA and DNA) in the algae of *Hydra viridis*, labeled hydras were washed 2–3 times with large volumes of cold "M" solution. The algae were separated from the animal fractions as described above, and the algal pellet further washed several times to remove any exogenous radioactivity. To 0.2 ml algal medium we added 0.2 ml of 6% acetic acid to fix the cells. Algae then were smeared on acid-washed albuminized slides and fixative allowed to evaporate. The slides were washed in distilled water, air dried, and stored at 4°C until they were autoradiographed with Kodak AR 10 stripping film. Test exposure of autoradiographed RNA slides was 21 days, and for DNA 93 days. Some slides were reserved for nuclease treatment before autoradiography. For DNA, spleen DNase II (Sigma Chemical Co., St. Louis Mo.) 1 mg/ml, buffered in McIlvaine's phosphate buffer at pH 6.8–6.9 in 0.002 *N* Mg SO_4, was incubated at 37°C for 4 h. For RNA, RNase (grade A from bovine pancreas) 1 mg/ml in McIlvaine's phosphate buffer, pH 6.7, was incubated at 37°C for 4 h. All slides were washed before being autoradiographed.

For incorporation of tritiated leucine, after several washes a 0.5 ml volume of animals was placed in 0.5 ml maceration solution (1:1:13 v/v/v glycerol:glacial acetic acid:distilled water) and fixed in this solution for 1 h. After fixation, a smear of the different cell types was air dried and autoradiographed. Exposure time was 21 days.

All autoradiographs were developed for 5 min in Kodak D-19 developer at 10–16°C, rinsed in Kodak stop bath, and then placed in Kodak rapid fix for 3 min. The slides were then rinsed in running water for 30 min. Algae grains were then counted by white light and phase contrast microscopy.

Results

Uptake of tritiated leucine by algae

Figure 1 shows the results of experiments in which algae were removed from hydras kept in the light and dark. The quantity of label in the algae under light and dark conditions fluctuated. Algae in hydras incubated in the dark took up more label than those in the light. However, due to inactivity of the algae, the label was not as efficiently used as in the light. Leakage or expulsion of algae could be responsible for the drastic drops at 72 and 240 h. In the light experiments, there were peaks at 12 h and again at 30 h. These might reflect times of increased algal activity. After 10 days in the dark, the algae still contained label.

Distribution of label in major fractions

Figure 2 (left) shows that most of the label in algae in hydras kept under constant light conditions was found in the small-molecule and protein fractions. Label in these fractions fluctuated very little over 72 h. The TCA soluble alcohol-insoluble labeled fraction (oligosaccharides, oligonucleotides) might represent intermediate metabolites. The trace amounts of label introduced as leucine in the nucleic acid fraction might be contamination from other fractions.

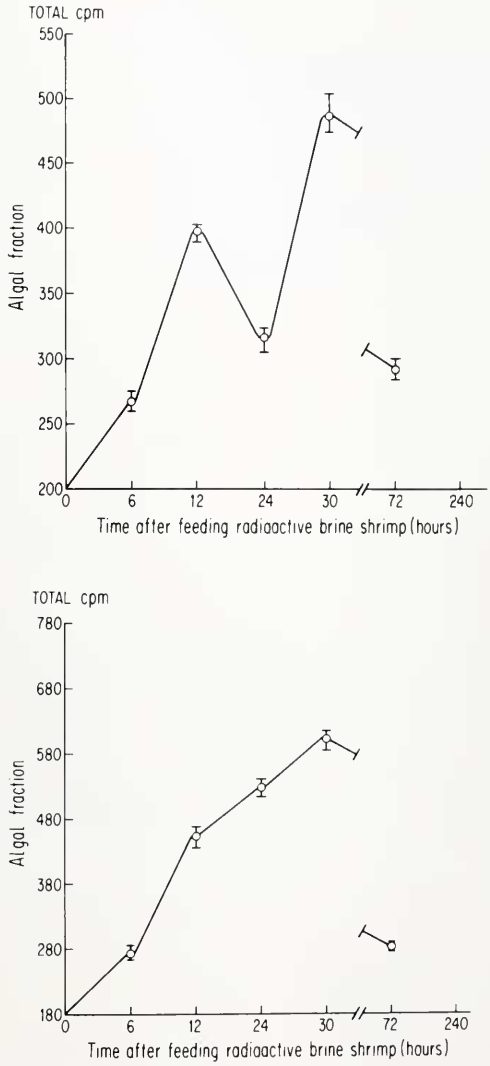


FIGURE 1. Uptake of tritiated leucine in algal fractions of light-grown hydras (above) and dark-grown hydras (below). Vertical bars show standard deviation. Each point shows means of total radioactivity counts of samples from four independent experiments, each using 60 hydras.

In the dark, label in the three major fractions just mentioned fluctuates more (Fig. 2, right). During the first six h, approximately 58% of the label was in the TCA-alcohol soluble fraction and about twice as much label was in the TCA-soluble-alcohol-insoluble fraction. Over the next 18 h, the radioactive material slowly changed, moving from the TCA-alcohol-soluble to the TCA-insoluble-fraction, indicating incorporation of amino acids into proteins. This continued about 30 h, after which the relative amounts of radioactivity in the small-molecule and protein fractions remained constant.

Neither in the light nor in the dark were lipid or lipid soluble compounds labeled, primarily because no lipid precursors were utilized. Since label introduced

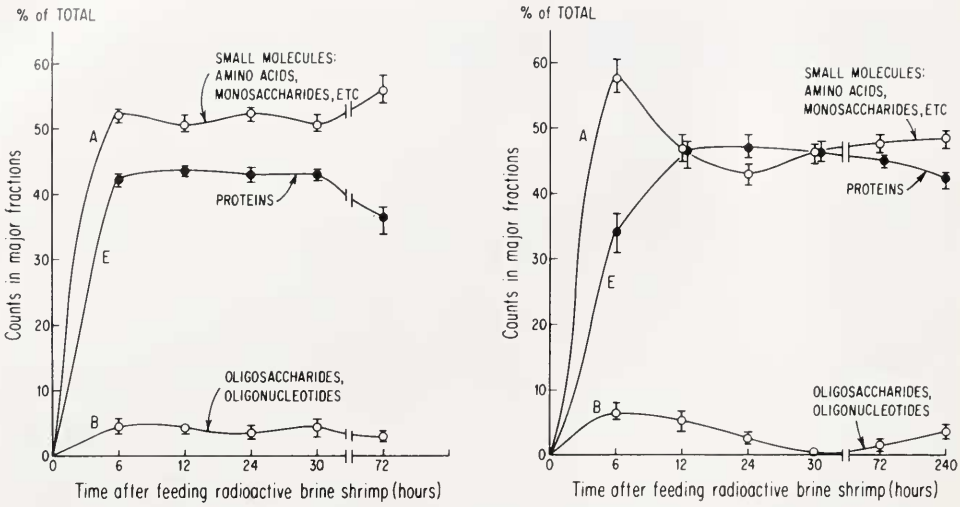


FIGURE 2. Tritiated leucine in major fractions of algae from light-grown (left) and dark-grown (right) hydras. Vertical bars show standard deviation. Each point shows the mean from four independent experiments, each using 60 hydras.

as leucine was seen in other fractions besides amino acids and proteins, it was being assimilated into algal metabolites. In the animal fraction from dark grown hydras at around 30 h label was incorporated into the TCA-insoluble alcohol-soluble lipid-soluble (lipid and lipid-soluble compounds; small protein) fraction. With longer time intervals, these compounds were lost either as a result of leakage or due to utilization by the organism. The ^{3}H-leucine data is summarized in Table 1. Figure 3 shows the distribution of label among the major biochemical fractions in the light- and dark-grown animal fractions.

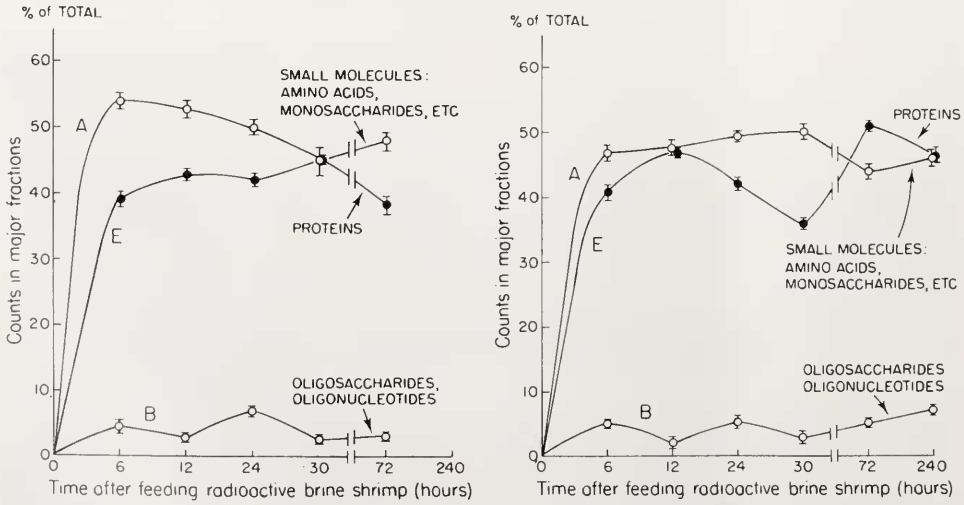


FIGURE 3. Tritiated leucine in major fractions from animal fraction from light-grown (left) and dark-grown (right) hydras. Vertical bars show the standard deviation. Each point is mean from four independent experiments, each using 60 hydras.

TABLE I

Algal and animal fraction ^{3}H-Leucine. Mean ± SD.

Fraction	6 h	12 h	24 h	30 h	72 h	10 days
Agal, light						
Amino acids & sugars	52 ± 0.64	50.8 ± 0.92	52.6 ± 1.14	50.2 ± 0.89	52.6 ± 0.89	—
Oligosaccharides & oligonucleotides	4.6 ± 0.86	4.5 ± 0.165	4.2 ± 1.15	4.8 ± 1.02	3.2 ± 0.70	—
Lipids	—	—	—	—	—	—
Nucleic acids	2.0 ± 0.96	1.16 ± 0.70	1.7 ± 0.96	1.8 ± 1.11	4.2 ± 1.19	—
Proteins	41.2 ± 0.90	43.4 ± 0.43	42.8 ± 1.3	42.7 ± 0.73	39.65 ± 1.95	—
Algal, dark						
Amino acids & sugars	57.8 ± 2.7	47 ± 2.0	42.9 ± 1.57	46.2 ± 1.42	48 ± 1.63	48.5 ± 1.02
Oligosaccharides & oligonucleotides	6.9 ± 1.48	5.2 ± 1.45	2.7 ± 0.58	0.53 ± 0.56	1.6 ± 0.70	3.8 ± 0.75
Lipids	—	—	—	—	—	—
Nucleic acids	0.76 ± 0.79	0.86 ± 0.84	7.36 ± 0.89	6.7 ± 1.15	4.9 ± 1.20	4.5 ± 0.56
Proteins	34.16 ± 2.9	46.5 ± 2.0	47.16 ± 1.67	46.2 ± 1.34	45.0 ± 0.75	42.5 ± 0.59
Animal, light						
Amino acids & sugars	54 ± 1.608	53.6 ± 1.34	50.3 ± 1.22	45.6 ± 2.29	48 ± 1.13	—
Oligosaccharides & oligonucleotides	4.4 ± 1.17	3.5 ± 1.12	6.7 ± 1.19	2.4 ± 0.31	3.3 ± 0.84	—
Lipids	—	—	—	—	—	—
Nucleic acids	1.8 ± 1.25	—	0.5 ± 0.53	5.9 ± 2.19	10.32 ± 1.05	—
Proteins	39 ± 1.50	42.7 ± 1.04	41.9 ± 1.69	45.5 ± 1.04	38.25 ± 1.41	—
Animal, dark						
Amino acids & sugars	46.6 ± 1.49	47.6 ± 0.33	48.9 ± 1.81	50 ± 1.73	44 ± 1.45	45.9 ± 0.27
Oligosaccharides & oligonucleotides	5.2 ± 1.19	2.0 ± 1.07	5.7 ± 1.42	3.4 ± 0.85	5.0 ± 0.53	7.1 ± 0.79
Lipids	0.3 ± 0.52	—	—	5.6 ± 1.55	—	—
Nucleic acids	6.6 ± 1.72	3.5 ± 1.44	5.2 ± 1.65	5.1 ± 1.45	0.2 ± 0.32	0.2 ± 0.38
Proteins	41 ± 1.08	46.6 ± 1.79	40 ± 1.47	35.6 ± 1.82	50.5 ± 1.57	46.4 ± 0.48

RNA precursors into algal RNA

Tritiated orotic acid and tritiated uridine, both RNA precursors, were taken up and incorporated into both animal and algal fractions (Fig. 4). Uptake of RNA precursors differed in algae obtained from light- and dark-grown animals (Fig. 4). In the algal fraction from light-grown hydras, less label delivered as H^3 uridine was incorporated into nucleic acid relative to label delivered as ^{3}H-orotic acid. However, after 30 h with both precursors about 86–89% of the label was in nucleic acid. Therefore, the ^{3}H-uridine is apparently accumulated by the algae but less readily incorporated into nucleic acid in the light than ^{3}H-orotic acid.

Slightly more ^{3}H-orotic acid was incorporated into nucleic acid, compared to the labeled uridine, in dark-grown hydras (Fig. 5). RNA precursors in algal macromolecules continued to increase for 30 h in the light and dark. So many variables affect these results that quantitative and direct comparison of precursors is inappropriate. For example, environmental conditions (such as temperature) and probably the rate at which various tissues used label varied.

Treatment with bovine pancreatic RNase reduced the total counts to background values, verifying that most of the label introduced as orotic acid or uridine was incorporated into RNA.

DNA precursors into algal DNA

The uptake of ^{3}H-thymidine in the insoluble fraction of the algae is shown in Figure 6. In light-grown hydras the algae are very active. They divide at a rate

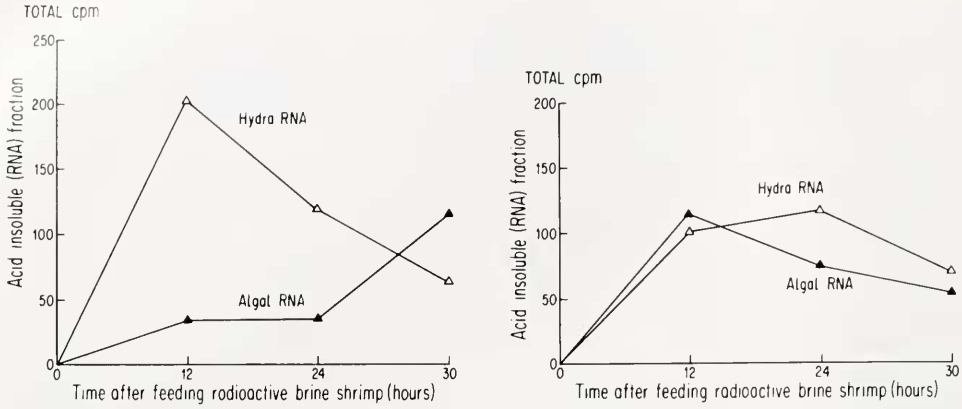
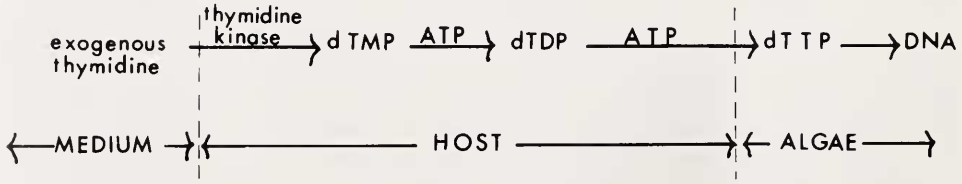


FIGURE 4. Tritiated uridine uptake and incorporation into RNA of animal and algal fractions from light-grown (left) and dark-grown (right) hydras. Each point is mean from four independent experiments, each using 60 hydras.

similar to that of cells of their hosts. The doubling time of green hydras is 2.0 days (Cook and Rupright, 1980). There was no statistically significant difference between the amounts of label incorporated into algae from light-grown and dark-grown hydras. With prolonged exposure, the quantity of labeled DNA decreased because of cell division.

According to Wanka *et al.* (1970), chlorellae lack the enzyme thymidine kinase. They take up, but degrade, the deoxynucleoside, and do not incorporate thymidine into DNA. In fact chlorellae utilize exogenous uridine more readily than thymidine as a precursor for DNA synthesis. They probably synthesize DNA by means of thymidylate synthetase. The symbiotic algal incorporation of thymidine into DNA seems to imply that the host kinases provide triphosphates which can then be utilized as well by the endosymbiotic chlorellae. Incorporation of thymidine into symbiotic algae DNA may be as indicated in this scheme:



Radioautography

Figure 7 shows a representative radioautograph, of algae isolated from light-grown hydras after 24 h in ^{3}H-leucine. The label was distributed over the isolated algae and the animal tissue.

Treatment of the cells with deoxyribonuclease and ribonuclease before autoradiography significantly decreased the amount of label, indicating that the labeling was due to incorporation into DNA or RNA respectively.

DISCUSSION

Cook (1972) suggested hydra "back transfer" of photosynthate to algae. Not only is carbon directly introduced as translocated bicarbonate across the vacuolar

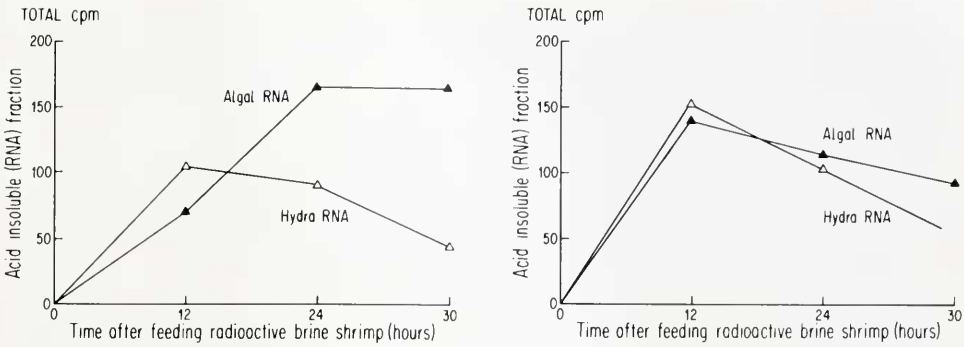
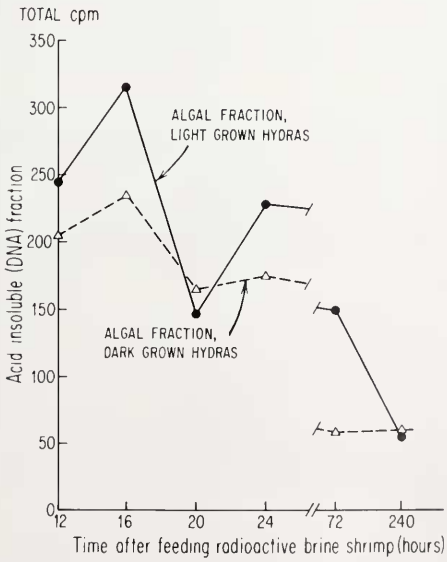


FIGURE 5. Tritiated orotic acid uptake and incorporation into RNA of animal and algal fractions from light-grown (left) and dark-grown (right) hydras. Each point is mean from four independent experiments, each using 60 hydras.

membranes of the symbionts, but protein precursors, nucleotide triphosphates, and ribonucleic acid precursors are also translocated as such. Labeled amino acid assimilated readily into algal and hydra proteins. It was also found in small molecules, such as amino acids, monosaccharides, oligosaccharides, and oligonucleotides. In hydras grown under constant light, there was little fluctuation in the small-molecule fraction, which incorporated most (about 52%) of the label. However, in dark-grown hydras, there was a correlation between the amount of label in the TCA-alcohol and soluble insoluble fractions. Amino acid incorporation into algae protein occurred, but with time hydrolysis of protein occurred as well. That is, the decrease



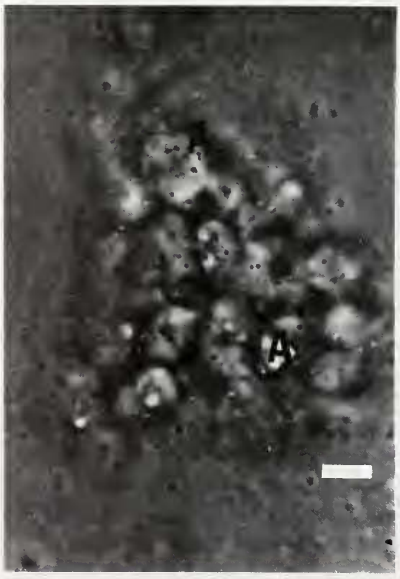


FIGURE 6. (Left) Tritiated thymidine uptake and incorporation into animal and algal fractions from light- and dark-grown hydras. Each point is mean from three independent experiments, each using 120 hydras.

FIGURE 7. (Right) Radioautograph of tritiated leucine labeled algae (A). Light micrograph (bar = 10 μm).

in label in the protein after 30 h corresponding to a rise in the other two fractions may be due to degradation of protein to supply energy for survival. The fractionation procedure did not elucidate whether the increase in the small molecule fraction reflected an increase in amino acids or in sugars.

Since photosynthesis was not occurring in the dark, and label appeared in the algal fractions, exogenous food (fed as brine shrimp to the host) was being hydrolyzed and taken up by the symbionts. The algae population in the gastrodermal cells decreased slightly after 10 days in darkness, from about 16 to about 11 algae per digestive cell. This suggests that on a per-cell basis, there was more incorporation in hydras maintained in the dark than in the light.

The differences in distribution of label between algae obtained from light- and dark-treated animals may reflect differences in the rate of assimilation of label or qualitative differences in metabolism of algae kept under these different conditions.

The conversion of label from the acid-soluble to the acid-insoluble fractions implied protein synthesis. The rate of conversion over 72 h in the animal fraction was correlated with that in the algal fraction in hydras incubated in the light. After 72 h, in both animal and algal light fractions, 40% of the label was in the form of protein. The correlation was less in dark-grown hydras.

Green hydras starved in the light derive about 75% of their energy from carbohydrates (Pardy and White, 1977), mainly by algal photosynthesis. Since in the present experiments the animals were fed only once, over the longer intervals used starvation probably set in. Although the animal's main energy source then would be photosynthetic products, some of the labeled products also may have been shunted into the carbohydrate metabolic pathway, thus supplying some of the host's metabolic needs (Fig. 3).

Thymidine was taken up by algal symbionts in both light- and dark-grown hydras. The total amount of label in both algal and host fractions is about twice as great in hydras incubated in the light than in the dark. The proportion of label in the animal fraction relative to the algal fraction remained high in hydras incubated in the light for 2 days, but by 3 days it decreased, presumably because of increased relative rate of host cell division.

Pardy (1974) reported a slight transient increase in growth rate of algae in hydra kept in the dark. Our studies showed increased uptake of thymidine in the algal fraction of dark-grown hydras at 16 h, probably reflecting algal growth. This was followed by a sharp decline in activity over the remaining 9½ days.

In the dark, algae may compete among themselves and with their hosts for metabolites (Pardy, 1974). This may slow hydra growth as much as 20%, and algae growth even more. Our observations seem consistent with Pardy's conclusions. Metabolites of brine shrimp (food) origin are available to both partners. A decrease in label in animal with time is compensated for by an increase of label in the algae. This is especially evident in ^{3}H-orotic acid and ^{3}H-uridine incorporation into RNA. Thus, host and algae may compete for constant quantities of metabolites from one feeding. Since we found no separate algal nucleotide pool, the algae probably have access to the hosts' ribonucleotide pool. In the dark, host cells contain more label relative to the algae cells. In fact, with prolonged darkness (7–10 days), the number of algae per cell is reduced. But in fed hydras, the symbiosis apparently persists indefinitely—or at least 3–4 months, until the experiment ended. Food availability and light influence the quantity and rate of nutrient transfer as well as the ratio of partner cells in this symbiosis.

As the label in the thoroughly washed algal pellet was in the algal fraction, and this was not due to contamination with animal tissue, "back transfer" of

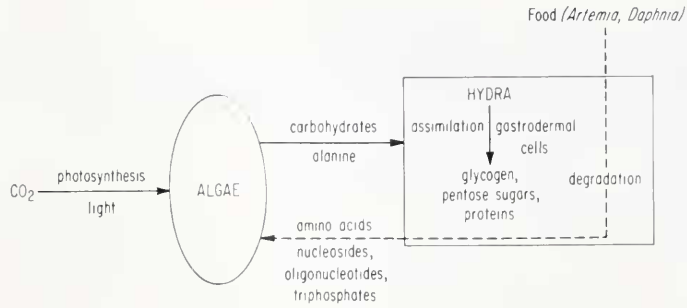


FIGURE 8. Nutrient exchange between hydra and symbiotic algae.

metabolites from the host to the algae occurs. From this source, dark-grown chlorellae obtain nutrients to metabolize and grow. Even in the light, large quantities of small food molecules are transferred to the autotrophs. Figure 8 summarizes the major routes of nutrient exchange between hydras and their symbiotic algae.

The bacterial symbionts of hydra probably also affect these metabolic interactions. Bacteria-free hydras double in 1.7 days as compared to the 2.0 days doubling time of green hydra with bacteria (Cook and Rupright, 1980). Our results and those of Wilkerson (1980) suggest that further quantitative studies of metabolic interactions in hydra symbioses must consider the roles of all three partners in nutrient flow: *Hydra viridis* (host), *Chlorella* sp. (symbiotic algae), and *Aeromonas punctata* (symbiotic bacteria) cells. The separability, manipulability, and experimentally achieved independent growth of the three partners in this symbiosis, coupled with the diverse quality and large quantity of metabolite flow, makes *Hydra viridis* and its microbes ideal for testing models of the evolutionary origin of symbioses (Smith, 1979).

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